CHEMISTRY LETTERS, pp. 817-820, 1987.

Ethylene Formation from 1-Aminocyclopropanecarboxylic Acid by the Reaction of Molecular Oxygen and Dihydropyridine Mediated by Flavin Mononucleotide and Mn(II) Ion#

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Oxidation of 1-aminocyclopropanecarboxylic acid by O_2 in the presence of 1-benzyl-3-carbamoyl-1,4-dihydropyridine, Mn(II) ion, and flavin mononucleotide reproduced the biological ethylene forming reaction in plant tissues with respect to products, stereochemistry, and behavior to inhibitors.

It is accepted that ethylene, a plant hormone controlling various physiological conditions of plants, is synthesized in plant cells by aerobic oxidation of 1-aminocyclopropanecarboxylic acid (ACC), the immediate precursor biosynthesized from *S*-adenosylmethionine.[1] Although extensive studies using plant tissues have established the stereochemical course of the reaction,[2,3] the details of the oxidation step have not been known because the enzyme is located in the cell membrane[4] and its isolation and purification have never been successful. Many cell free systems of biological origins have been reported as the biomimetic models,[5] but these systems were mixtures of several compounds, which made the evaluation of the results not straightfoward. In fact, a recent study showed that a potential model system consisting of lipoxygenase—pyridoxal phosphate —Mn(II) —O_2[6] has been shown to be different from the ethylene forming enzyme in plant tissues on the basis of the responces to inhibitors.[5] In this context, a chemical model relating to the biological reaction is helpful for better understanding of the oxidation step itself. We report in this communication an effective chemical system of ethylene synthesis from 1-aminocyclopropanecarboxylic acid composed of 1-benzyl-3-carbamoyl-1,4-dihydropyridine (BNAH)—Mn(II)—flavin mononucleotide (FMN)—O_2. This system reproduced some characteristics of the biological oxidation of ACC in plant tissues.

The model reaction was carried out in a buffer solution at 23 °C in the dark. The reaction was initiated by addition of Mn(II) solution to the mixture of ACC, BNAH, and FMN, and incubated under O_2 atmosphere. The products identified were ethylene (determined by gas chromatography), CN^- ion (determined by cyanide electrode), and carbon dioxide (not quantitatively determined, but its presence in a large amount after the reaction was identified

#This paper is dedicated to the late Professor Ryozo Goto, Kyoto University.

Table 1. Oxidation of ACC with Molecular Oxygen[a)]

Oxidizing system	pH	Product yield/%[b)]	
		Ethylene	CN^-
FMN—BNAH—Mn(II)	9.0	58.0	22.1
BNAH—Mn(II)	9.0	23.0	—[c)]
FMN—BNAH	9.0	13.3	13.0
FMN—Mn(II)	9.0	2.5	2.7
Mn(II)	9.0	0.4	—[c)]
FMN—BNAH—Mn(II)	4.5	3.0	1.9
FMN—BNAH—Mn(II)	7.4	39.0	34.5
FMN—BNAH—Mn(II)—SOD	7.5	56.5	32.0
FMN—BNAH—Mn(II)—Catalase	7.5	6.1	6.2
FMN—BNAH—Mn(II)—PrGal[d)]	7.4	7.0	—[c)]
FMN—BNAH—Mn(II)—AOAc[d)]	7.4	18.2	—[c)]
FMN—BNAH—Mn(II)—Catalase—PrGal[d)]	7.4	2.5	—[c)]
FMN—BNAH—Mn(II)—Catalase—AOAc[d)]	7.4	5.8	—[c)]

a) Reaction conditions: ACC, 5×10^{-2} M; BNAH, 5×10^{-2} M; FMN, 7×10^{-3} M; Mn(II), 8×10^{-3} M; Catalase, 3000 unit; SOD, 1000 unit; inhibitors, 1.4×10^{-2}M; (1 M = 1 mol dm^{-3}) 17 h, 23 °C in borate buffer. *b*) Yields were calculated on the used ACC basis. *c*) Not determined. *d*) Abbreviations: PrGal, propyl gallate; AOAc, aminooxyacetic acid (H_2NOCH_2COOH).

by gas chromatography-MS and IR spectra), similarly as those of the reactions *in vivo*. Molecular oxygen, Mn(II) ion, and a dihydropyridine were essential for the catalytic reaction as shown in Table 1. The reaction was pH-dependent, proceeding with a faster rate at higher pH. The amount of CN^- ion identified was low compared with the amount of ethylene, especially at high pH due to the decomposition under the reaction conditions.

The effects of additives, such as inhibitors and accelerators were also included in Table 1. Addition of superoxide dismutase (SOD) increased the yield of ethylene. But catalase showed a strong inhibiting effect to the reaction. About 90% of the model reaction was suppressed in the presence of catalase. However, even in the presence of various amounts of catalase, about 10% of reaction was not inhibited and the amount of CN^- ion produced was in good agreement with that of ethylene. These results suggest that H_2O_2 is involved in the ethylene forming reaction and in the nonenzymatic decomposition of CN^-. In fact, a stoichiometric amount of H_2O_2 induced efficient conversion of ACC to ethylene and also decomposition of CN^- ion in the presence of Mn(II). Contrary to the lipoxygenase system mentioned above,[6)] some inhibitors of ethylene forming enzyme such as propyl gallate and aminooxyacetic acid were also effective in this reaction, although the effect of the latter was small.

Stereochemical course of the reaction was studied by using (*E*)- and (*Z*)-ACC-2,3-d_2 as the substrates, which were prepared according to the procedure of O'Donnell from 1,2-dibromoethane-d_2,[7)] and by determining the distribution of

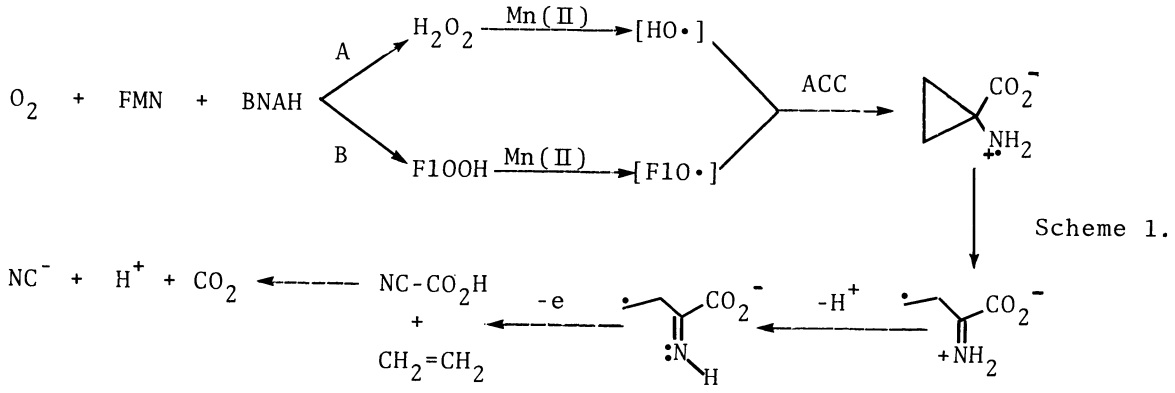
Table 2. Stereochemistry of the Reaction of ACC-d_2[a)]

Substrate	Catalase	pH	Product distribution/%	
			(Z)-CHD=CHD	(E)-CHD=CHD
(E)-ACC-2,3-d_2	no	7.4	47.9	52.1
(E)-ACC-2,3-d_2	no	11.1	51.0	49.0
(E)-ACC-2,3-d_2	yes	7.5	49.2	50.8
(E)-ACC-2,3-d_2	yes	11.1	50.5	49.5
(E)-ACC-2,3-d_2[b)]	—	12.0	100	0
(Z)-ACC-2,3-d_2	no	7.4	51.1	48.9
(Z)-ACC-2,3-d_2	no	11.0	52.8	47.2
(Z)-ACC-2,3-d_2	yes	7.5	49.7	50.3
(Z)-ACC-2,3-d_2	yes	11.4	50.3	49.7
(Z)-ACC-2,3-d_2[b)]	—	12.0	0	100

a) Reaction conditions were the same as in Column 9, Table 1. b) Reaction with NaOCl.

(E)- and (Z)-ethylene-d_2 with FTIR for comparison with the reaction *in vivo*.[8] Both in the presence and absence of catalase, a 1:1 mixture of two stereoisomers was formed in the reaction, indicating the nonstereoselective nature of oxidation similar to that of the enzyme. The results are summarized in Table 2.

The reaction course is proposed in Scheme 1. The formation of H_2O_2 and 4-hydroperoxyflavin (FlOOH) from FMN and O_2 in the presence of reductants such as dihydropyridines are well known.[9] These peroxides are decomposed by Mn(II) ion *via* a Fenton-like reaction.[10] Catalase should inhibit the reaction of H_2O_2 (route A), but it might not interfere with that of FlOOH (route B). Radical (RO·) induced ethylene formation has precedents in the oxidation of ACC.[5,11]



Scheme 1.

Another potential route of catalase-uninhibited reaction is the direct electron transfer reaction between the oxidized form of FMN and ACC with formation of $ACC^{\cdot+}$ cation radical.[12] This possibility was examined by the control reaction using an equimolar amount of FMN in the absence of BNAH. The yield of ethylene under the reaction conditions was only 0.3%, small enough to

eliminate the possibility in this reaction. By the way, a water soluble manganese porphyrin complex showed no catalytic activity in the presence of BNAH and O_2. This result can be correlated to the inhibition of cytochrome P-450 catalysis by cyclopropyl amine through the electron-transfer reaction from amine, and subsequent destruction of the catalyst.[13)]

Thus, the presented system has similarities with the ethylene forming enzyme *in vivo* with respect to the formation of ethylene, CN^-, and carbon dioxide, nonstereoselectivity of the product, and some behavior toward inhibitors. Although stereoselection of the substrate[3)] is not expectative with this system, asymmetric selection can be expected in biological systems since flavin is usually bound to chiral protein.

References

1) D. O. Adams and S. F. Yang, Proc. Natl. Acad. Sci. U.S.A., 76, 170 (1979).
2) M. C. Pirrung, J. Am. Chem. Soc., 105, 7207 (1983); M. C. Pirrung and G. M. McGeehan, J. Org. Chem., 51, 2103 (1986); R. M. Adlington, J. E. Baldwin, and B. J. Rawlings, J. Chem. Soc., Chem. Commun., 1983, 290; J. E. Baldwin, D. A. Jackson, R. M. Adlington, and B. J. Rawlings, *ibid.*, 1985, 206; J. E. Baldwin, R. M. Adlington, G. A. Lajoie, and B. J. Rawlings, *ibid.*, 1985, 1496.
3) N. E. Hoffman, S. F. Yang, A. Ichihara, and S. Sakamura, Plant Physiol., 70, 195 (1982).
4) J. R. Konze and H. Kende, Planta, 146, 293 (1979); J. R. Konze, J. F. Jones, T. Boller, and H. Kende, Plant Physiol., 66, 566 (1980).
5) J.-F. Bousquet and K. V. Thimann, Proc. Natl. Acad. Sci. U.S.A., 81, 1724 (1984) and references cited therein.
6) M. C. Pirrung, Biochemistry, 25, 114 (1986).
7) M. J. O'Donnell, W. A. Bruder, T. M. Eckrich, D. F. Schullenberger, and G. S. Staten, Synthesis, 1984, 127.
8) The ratio of (*E*)- and (*Z*)-ethylene-d_2 was determined with reference to the absorptions at 841 cm^{-1} ((*Z*)-isomer) and 989 cm^{-1} ((*E*)-isomer).
9) T. C. Bruice, Isr. J. Chem., 24, 54 (1984) and references cited therein.
10) When Fe(II) ion was used instead of Mn(II), the catalytic efficiency was low and dark precipitate was observed during the reaction. The results can be correlated to the low solubility of iron species under the reaction conditions.
11) R. L. Legge, J. E. Thompson, and J. E. Baker, Plant Cell Physiol., 23, 171 (1982).
12) R. B. Silverman, S. J. Hoffman, and W. B. Catus III, J. Am. Chem. Soc., 102, 7126 (1980); R. B. Silverman and S. J. Hoffman, Biochem. Biophys. Res. Commun., 101, 1396 (1981).
13) T. L. Macdonald, K. Zirvi, L. T. Burka, P. Peyman, and F. P. Guengerich, J. Am. Chem. Soc., 104, 2050 (1982); R. P. Hanzlik and R. H. Tullman, ibid., 104, 2048 (1982).

(Received January 30, 1987)